

FIG. 11

Signal to noise ratio of ELISA
 reading using phospho-Serine/threonine
 phenylalanine antibody.

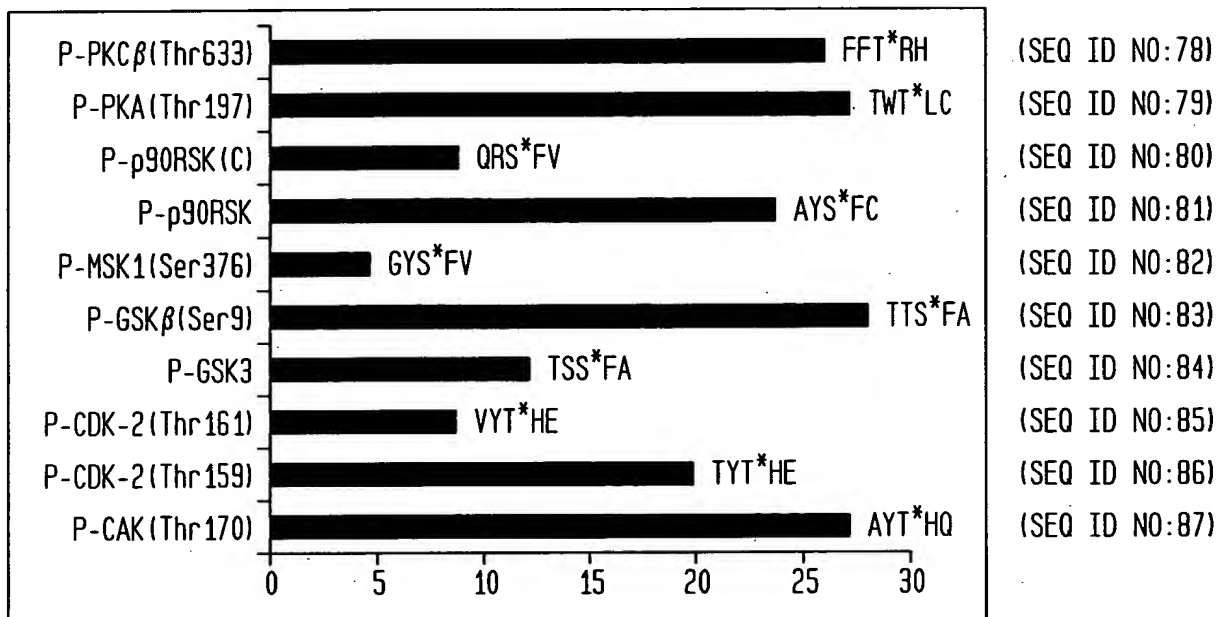
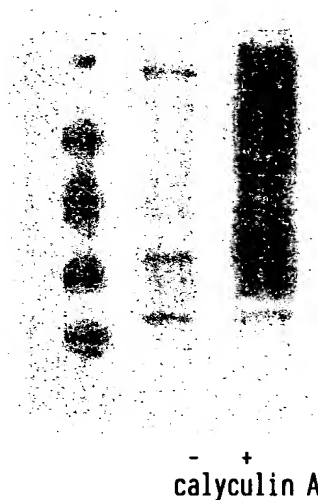


FIG. 12

Western analysis of calyculin
A-treated A431 cells using phospho-
Serine/phenylalanine substrates antibody.



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FIG. 7

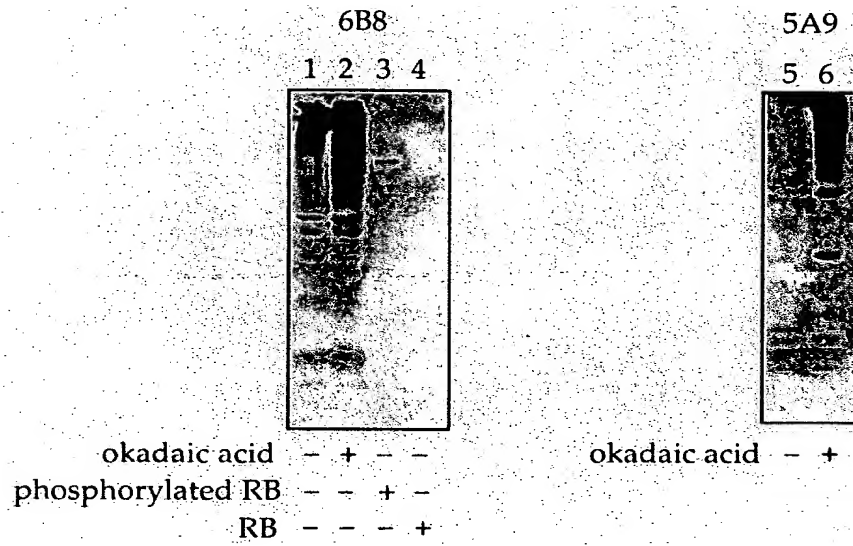
Western analysis of
calyculin A-treated A431 cells
using Phospho-Akt Substrate Anti-
body.



- +
calyculin A

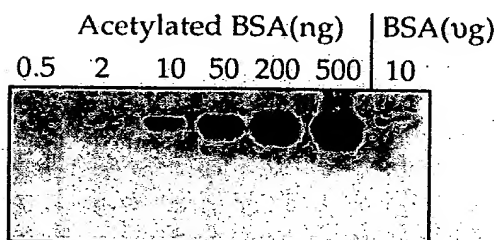
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FIG. 4B



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FIG. 5A



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FIG. 5B



FIG. 5C



FIG. 5D



FIG. 3B

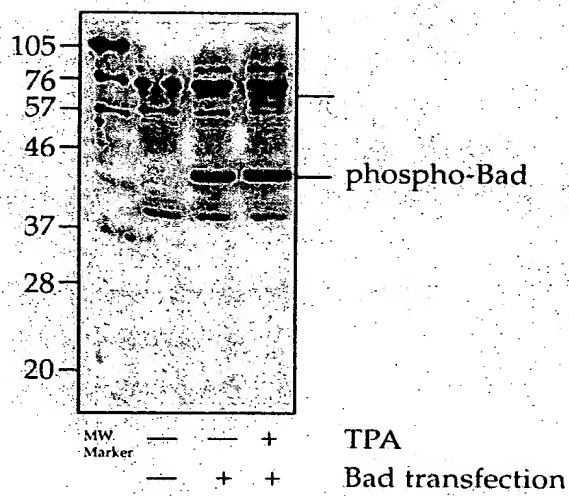


FIG. 2B

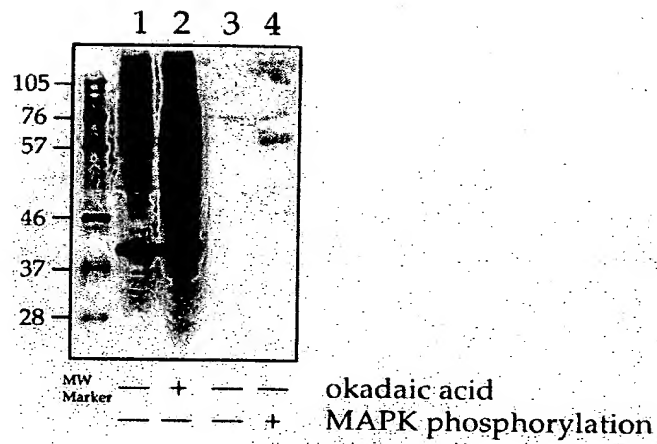




FIG. 9

Western analysis of
calyculin A-treated A431 cells
using Phospho-PKA Substrate
Antibody.

- + calyculin A

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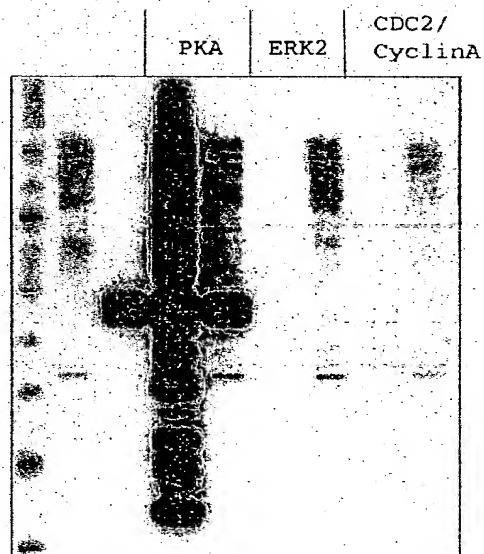


FIG. 10

Western analysis of A431 cell extracts

$$\begin{array}{cccccccc} + & - & + & + & - & + & - & + \\ - & - & - & + & - & - & - & - \end{array}$$

Cell Extracts
PKI

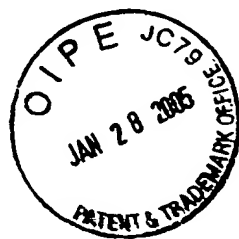


FIG. 1A

PEPTIDE	SEQUENCE	1.00E+03	ANTIBODY DILUTIONS				1.00E+06
			5.00E+03	1.00E+04	5.00E+04	1.00E+05	5.00E+06
Thr*	X-X-X-X-X-Thr-X-X-X-X-X-X-Cys	1.92	1.32	0.54	0.34	0.07	0.04
Ser-Thr	X-X-X-X-X-Ser-Thr-X-X-X-X-X-X-Cys	0.11	0.05	0.01	0.00	0.01	0.00
Threonine* mix	18 phospho-Thr peptide	1.84	1.13	0.40	0.26	0.10	0.07
Serine* mix	38 phospho-Ser peptide	0.12	0.04	0.02	0.02	0.02	0.01
Akt-Thr308-P	Ile-Lys-Asp-Gly-Ala-Thr-Met-Lys-Thr-Phe-Cys-Gly-Thr-Pro (SEQ ID NO:1)	1.18	0.65	0.24	0.13	0.03	0.01
APPI-Thr688-P	Asp-Ala-Ala-Val-Thr-Pro-Lys-Lys-Arg-His-Leu-Ser-Lys-Cys (SEQ ID NO:2)	0.14	0.03	0.01	0.01	0.01	0.01
C3-P	Asp-Thr-Gln-Ile-Lys-Arg-Aer-Thr-Phe-Val-Gly-Thr-Pro-Phe-Cys (SEQ ID NO:3)	1.71	1.13	0.39	0.22	0.03	0.02
CAK-Thr167-P	His-Gln-Val-Val-Thr-Arg-Tyr-Tyr-Arg-Cys (SEQ ID NO:4)	1.77	1.15	0.41	0.27	0.06	0.03
CAMIV-Thr188-P	His-Gln-Val-Leu-Met-Lys-Thr-Val-Cys-Gly (SEQ ID NO:5)	1.79	1.36	0.63	0.40	0.08	0.05
CDC2-Thr167-P	Ile-Pro-Ile-Arg-Val-Thr-Thr-His-Glu-Val-Val-Thr-Leu-Cys (SEQ ID NO:6)	1.02	0.56	0.14	0.08	0.03	0.01
CDK2-Thr158-P	Gly-Val-Pro-Val-Arg-Thr-Thr-His-Glu-Val-Val-Thr-Leu-Cys (SEQ ID NO:7)	1.88	1.79	0.51	0.44	0.08	0.04
p70S8K-Thr389-P	Aer-Gln-Val-Phe-Leu-Gly-Phe-Thr-Tyr-Val-Ala-Pro-Lys-Lys-Cys (SEQ ID NO:8)	1.99	1.44	0.62	0.39	0.08	0.04
PKCalpha-P	Lys-Glu-His-Met-Met-Asp-Gly-Val-Thr-Thr-Arg-Thr-Phe-Cys (SEQ ID NO:9)	1.62	1.63	0.94	0.58	0.15	0.08
ERK2-P	Asp-His-Thr-Gly-Phe-Leu-Thr-Glu-Tyr-Val-Ala-Thr-Arg-Tyr-Cys (SEQ ID NO:10)	1.56	1.18	0.51	0.30	0.07	0.04
Myc Ser58/62-P	Glu-Leu-Leu-Pro-Thr-Pro-Pro-Leu-Ser-Pro-Ser-Arg-Ser-Cys (SEQ ID NO:11)	0.11	0.05	0.03	0.02	0.02	0.02
P38-2P	Leu-Ala-Arg-His-Thr-Asp-Glu-Met-Thr-Gly-Tyr-Val-Ala-Thr-Arg-Cys (SEQ ID NO:12)	0.54	0.30	0.08	0.08	0.04	0.04
JNK-2P	Ser-Phe-Met-Met-Thr-Pro-Tyr-Val-Val-Thr-Arg-Tyr-Tyr-Arg-Cys (SEQ ID NO:13)	1.49	0.44	0.12	0.07	0.03	0.02

FIG. 1B

PEPTIDE SEQUENCE	phospho-Thr Reactivity
XXXXXXS*XXXXXX	—
XXXXY*XXXX	—
XXXXXPXS*/T*PXR/KXXX (SEQ ID NO:14)	+ +
XXXXRSXS*XPXXXX (SEQ ID NO:15)	—
XXXXRSXSXPXXXX (SEQ ID NO:16)	—
XXXXXPXS*/T*PXXXXX (SEQ ID NO:17)	+ +
XXXXXPXS/TPXXXXX (SEQ ID NO:18)	—
XXXXXT*XXXXXX	+ + +
XXXXXXS/TXXXXXX	—
21 phospho-Thr peptides mixture	+ + +
38 phospho-Ser peptides mixture	—
30 phospho-Tyr peptides mixture	—
NEB LIBRARY	
X-X-X-X-D/E-X-X-S*-T*-X-X-X-X-X-C (SEQ ID NO:19)	+ + +
X-X-X-X-X-X-S*/T*-D/E-D/E-D/E-X-X-X (SEQ ID NO:20)	+ +
X-X-X-X-F-X-X-F-S*/T*-F/Y-X-X-X-X-C (SEQ ID NO:21)	+ + +
X-X-X-X-R/K-X-S*/T*-X-X-X-X-X-X-C (SEQ ID NO:22)	+ + +
X-X-X-R/K-X-X-S*/T*-X-X-X-X-X-X-C (SEQ ID NO:23)	+ + +
X-X-X-X-X-X-S*/T*-F/I/M-X-X-X-X-X-C (SEQ ID NO:24)	+ + +
X-X-X-X-X-X-S*/T*-F/I-X-X-X-X-X-C (SEQ ID NO:25)	+ + +
X-X-X-X-X-X-S*/T-P-X-X-X-X-X-X-C (SEQ ID NO:26)	+ +
X-X-X-X-X-T*-X-X-X-X-X-X-C	+ + +
X-X-X-X-X-P-X-S*/T*-P-X-X-X-X-X-C (SEQ ID NO:27)	+ +
X-X-X-X-X-X-S/T-X-X-X-X-X-X-C (SEQ ID NO:28)	—
X-X-X-X-X-P-X-S*/T*-P-X-R/K-X-X-X-C (SEQ ID NO:29)	+ +
ANTIBODY REACTIVITY	
+ + + very strong	ELISA O.D. > 2
+ + strong	1 - 2
+ weak	0.2 - 1
- very little	< 0.2

FIG. 1D

Fixed Amino Acid	-5-4-3-2-1 +1+2+3+4+5 X X X X X Ser*/Thr* X X X X X							
	Fixed AA position relative to phospho-Ser*/Thr*							
	- 4	- 3	- 2	- 1	S*/T*	+ 1	+ 2	+ 3
Ala	+	+	+	+		+	+	+
Cys	+	+	+	+		+	+	+
Asp	+	+	+	+		+	+	+
Glu	+	+	+	+		+	+	+
Phe	+	+	+	+		+	+	+
Gly	+	+	+	+		+	+	+
His	+	+	+	+		+	+	+
Ile	+	+	+	+		+	+	+
Lys	+	+	+	+		+	+	+
Leu	+	+	+	+		+	+	+
Met	+	+	+	+		+	+	+
Asn	+	+	+	+		+	+	+
Pro	+	+	+	+		—	+	+
Gln	+	+	+	+		+	+	+
Arg	+	+	+	+		+	+	+
Ser	+	+	+	+		+	+	+
Thr	+	+	+	+		+	+	+
Val	+	+	+	+		+	+	+
Trp	+	+	+	+		+	+	+
Tyr	+	+	+	+		+	+	+

FIG. 2A

PEPTIDE	SEQUENCE	ANTIBODY DILUTIONS					1.00E+03	5.00E+03	1.00E+04	5.00E+04	1.00E+05	5.00E+05	1.00E+06
PXSP-P	X-X-X-X-Pro-X-Ser-Thr-Pro-X-X-X-X-Cys (SEQ ID NO: 27)		1.82	1.97	1.74	1.40				0.70	0.35		0.08
Threonine mix	18 phospho-Thr peptide mix		1.97	1.37	0.67	0.36				0.13	0.07		0.05
Ser/Thr	X-X-X-X-X-Ser-Thr-X-X-X-X-X-Cys (SEQ ID NO: 28)		0.14	0.03	0.01	0.00				0.00	0.00		0.00
RB Thr373-P	Val-Ile-Pro-Pro-His-Thr-Pro-Val-Aug-Thr-Val-Met-Asn-Thr-Cys (SEQ ID NO: 30)		2.07	2.17	1.70	1.20				0.48	0.18		0.03
MKK3-Thr-P	Ser-Val-Ala-Lys-Thr-Met-Asp-Ala-Gly-Cys (SEQ ID NO: 31)		0.06	0.04	0.01	0.00				0.00	0.00		0.00
PKCalpha-P	Lys-Glu-His-Met-Met-Asp-Gly-Val-Thr-Thr-Arg-Thr-Phe-Cys (SEQ ID NO: 9)		0.05	0.02	0.01	0.00				0.01	0.00		0.00
p70 S6K-Thr388	Asn-Gln-Val-Phe-Leu-Gly-Phe-Thr-Tyr-Val-Ala-Pro-Lys-Lys-Cys (SEQ ID NO: 8)		0.11	0.05	0.01	0.00				0.01	0.00		0.00
cdk4-Thr172-P	Arg-Ile-Tyr-Ser-Tyr-Gln-Met-Ala-Leu-Thr-Pro-Val-Val-Lys-Cys (SEQ ID NO: 32)		2.07	2.21	2.01	1.55				0.69	0.31		0.07

FIG. 3A

PEPTIDE	SEQUENCE	1.00E+03	5.00E+03	ANTIBODY DILUTIONS			1.00E+05	5.00E+05
				1.00E+04	5.00E+04	1.00E+05		
14-3-3 BM-P	X-X-X-X-Arg-Ser-X-Ser-X-Pro-X-X-X-X-Cys (SEQ ID NO:33)	2.41	2.15	1.49	1.15	0.44	0.25	
14-3-3 BM	X-X-X-X-Arg-Ser-X-Ser-X-Pro-X-X-X-X-Cys (SEQ ID NO:34)	0.07	0.03	0.02	0.03	0.02	0.03	
CDC25-Ser216-P	Gly-Leu-Tyr-Arg-Ser-Pro-Ser-Met-Pro-Glu-Asn-Leu-Asn-Arg-Cys (SEQ ID NO:35)	2.35	2.08	1.49	1.05	0.33	0.18	
CDC25-Ser216	Gly-Leu-Tyr-Arg-Ser-Pro-Ser-Met-Pro-Glu-Asn-Leu-Asn-Arg-Cys (SEQ ID NO:36)	0.05	0.02	0.03	0.03	0.04	0.03	
Bad-Ser112-P	Thr-Arg-Ser-Arg-His-Ser-Ser-Tyr-Pro-Ala-Gly-Thr-Glu-Glu-Cys (SEQ ID NO:37)	1.59	0.43	0.10	0.03	0.01	0.00	
Bad-Ser112	Thr-Arg-Ser-Arg-His-Ser-Ser-Tyr-Pro-Ala-Gly-Thr-Glu-Glu-Cys (SEQ ID NO:38)	0.00	0.00	0.00	0.00	0.00	0.00	
Bad-Ser136	Phe-Arg-Gly-Arg-Ser-Arg-Ser-Ala-Pro-Pro-Asn-Leu-Trp-Ala-Cys (SEQ ID NO:39)	0.03	0.00	0.00	0.00	0.00	0.00	
Bad-Ser136-P	Phe-Arg-Gly-Arg-Ser-Arg-Ser-Ala-Pro-Pro-Asn-Leu-Trp-Ala-Cys (SEQ ID NO:40)	3.25	1.86	0.73	0.51	0.07	0.03	

FIG. 4A

PEPTIDE	SEQUENCE	MONOCLONAL ANTIBODIES	
		6B8	5A9
Ser/ThrPro-P	X-X-X-X-X-Ser/Thr-Pro-X-X-X-X-X-Cys (SEQ ID NO:26)	1.774	0.731
ProXSer/ThrPro-P	X-X-X-X-X-Pro-X-Ser/Thr-Pro-X-X-X-X-X-Cys (SEQ ID NO:27)	0.924	0.766
ProXSer/ThrPro-P	X-X-X-X-X-Pro-X-Ser/Thr-Pro-X-X-X-X-X-Cys (SEQ ID NO:41)	0.02	0.063
ProXSer/ThrProXArg-P	X-X-X-X-X-Pro-X-Ser/Thr-Pro-X-Arg/Lys-X-X-X-Cys (SEQ ID NO:42)	1.955	1.275
Thr-P	X-X-X-X-X-Thr-X-X-X-X-X-Cys	0	--
Ser-P	X-X-X-X-X-Ser-X-X-X-X-X-Cys	0.031	0.088
Ser/Thr	X-X-X-X-X-Ser/Thr-X-X-X-X-X-Cys	0.021	0.066
Tyr-P	X-X-X-X-X-Tyr-X-X-X-X-X-Cys	0.023	0.072
Rb (Ser795)-P	Ser-Pro-Tyr-Lys-Phe-Pro-Ser-Ser-Pro-Leu-Arg-Ile-Pro-Gly-Cys (SEQ ID NO:43)	0.032	0.124
Rb (Thr373)-P	Val-Ile-Pro-Pro-His-Thr-Pro-Val-Arg-Thr-Val-Met-Asn-Thr-Cys (SEQ ID NO:30)	3.336	3.503
Rb (Thr373)	Val-Ile-Pro-Pro-His-Thr-Pro-Val-Arg-Thr-Val-Met-Asn-Thr-Cys (SEQ ID NO:44)	0.02	0.073

FIG. 6

Signal to noise ratio of ELISA
readings using phospho-Akt substrate
antibody.

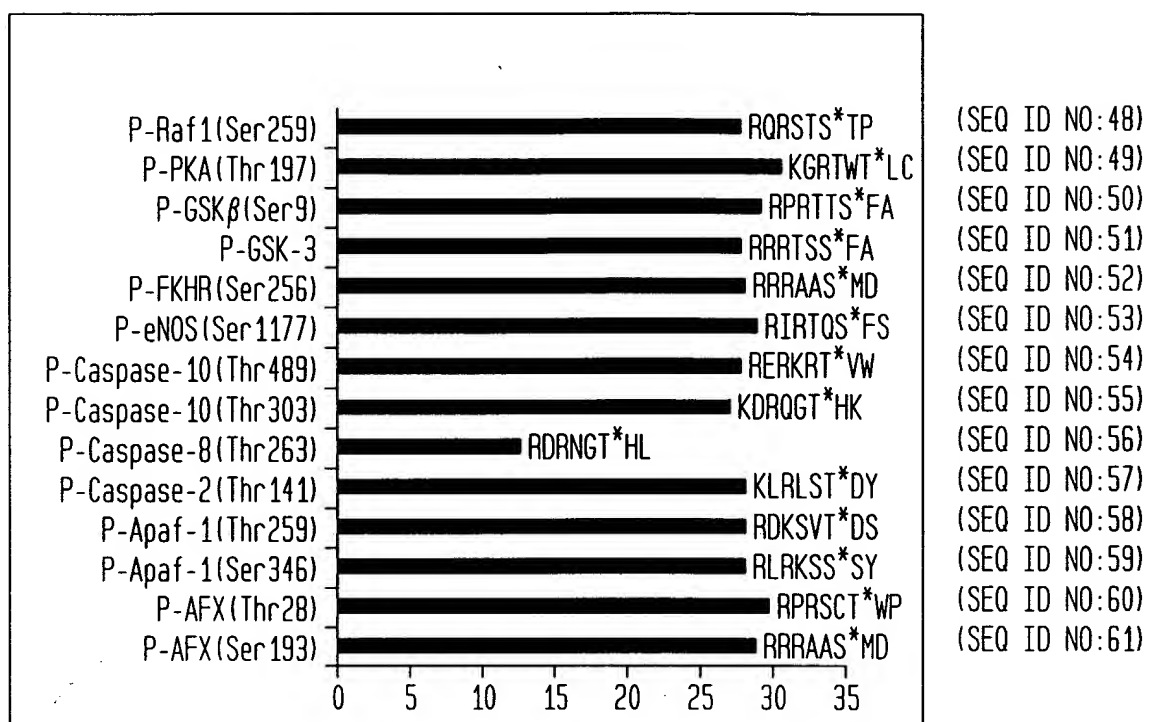


FIG. 8

Signal to noise ratio of ELISA
reading using phospho-PKA substrates
antibody.

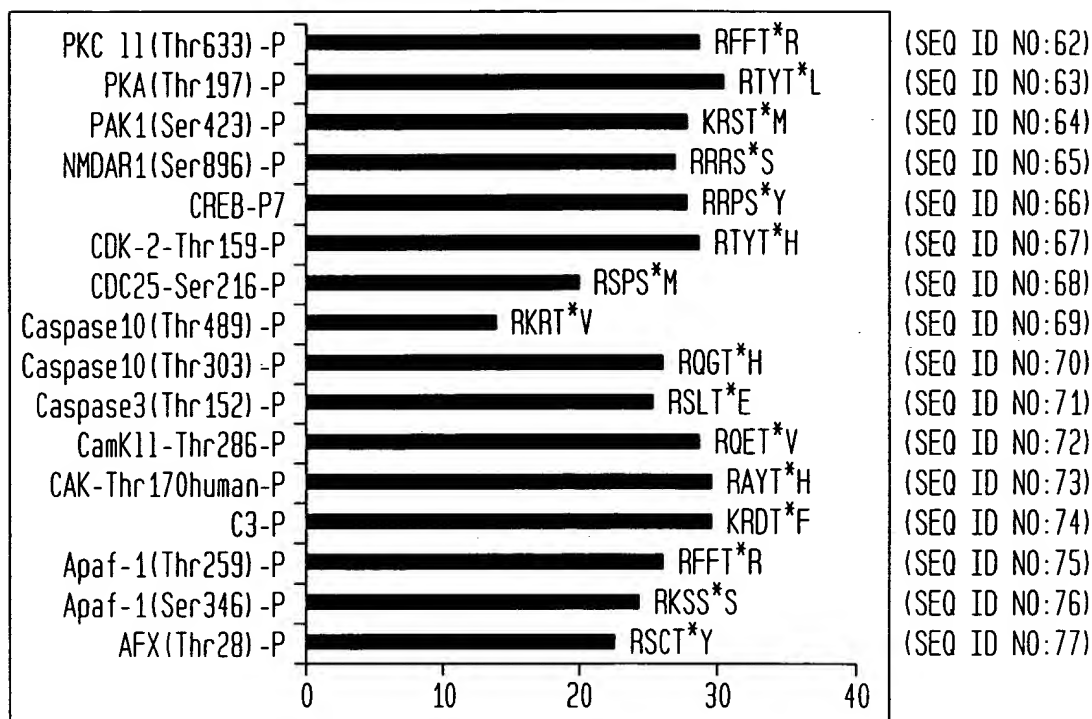




FIG. 1A

PEPTIDE	SEQUENCE	ANTIBODY DILUTIONS					
		1.00E+03	5.00E+03	1.00E+04	5.00E+04	1.00E+05	5.00E+05
Thr	X-X-X-X-Thr-X-X-X-X-X-X-Cys	1.82	1.32	0.54	0.34	0.07	0.04
Ser-Thr	X-X-X-X-X-Ser-Thr-X-X-X-X-X-X-Cys	0.11	0.05	0.01	0.00	0.01	0.00
Threonine mix	18 phospho-Thr peptide	1.84	1.13	0.40	0.28	0.10	0.07
Serine mix	38 phospho-Ser peptide	0.12	0.04	0.02	0.02	0.02	0.01
Akt-Thr808-P	Ile-Lys-Asp-Gly-Ala-Thr-Met-Lys-Thr-Phe-Cys-Gly-Thr-Pro (SEQ ID NO:1)	1.18	0.65	0.24	0.13	0.03	0.01
APP1-Thr668-P	Asp-Ala-Ala-Val-Thr-Pro-Lys-Lys-Arg-His-Leu-Ser-Lys-Cys (SEQ ID NO:2)	0.14	0.03	0.01	0.01	0.01	0.01
C3-P	Asp-Thr-Gln-Ile-Lys-Arg-Asn-Thr-Phe-Val-Gly-Thr-Pro-Phe-Cys (SEQ ID NO:3)	1.71	1.13	0.39	0.22	0.03	0.02
CAK-Thr167-P	His-Gln-Val-Val-Thr-Arg-Trp-Tyr-Arg-Cys (SEQ ID NO:4)	1.77	1.15	0.41	0.27	0.06	0.03
CAMIV-Thr188-P	His-Gln-Val-Leu-Met-Lys-Thr-Val-Cys-Gly (SEQ ID NO:5)	1.79	1.36	0.63	0.40	0.08	0.05
CDC2-Thr167-P	Ile-Pro-Ile-Arg-Val-Tyr-Thr-His-Glu-Val-Val-Thr-Leu-Cys (SEQ ID NO:6)	1.02	0.56	0.14	0.08	0.03	0.01
CDK2-Thr159-P	Gly-Val-Pro-Val-Arg-Thr-Tyr-Thr-His-Glu-Val-Val-Thr-Leu-Cys (SEQ ID NO:7)	1.88	1.79	0.51	0.44	0.08	0.04
p70S6K-Thr389-P	Asn-Gln-Val-Phe-Leu-Gly-Phe-Thr-Tyr-Val-Ala-Pro-Lys-Lys-Cys (SEQ ID NO:8)	1.89	1.44	0.62	0.39	0.08	0.04
PKCalpha-P	Lys-Glu-His-Met-Met-Asp-Gly-Val-Thr-Thr-Arg-Thr-Phe-Cys (SEQ ID NO:9)	1.82	1.63	0.94	0.58	0.15	0.08
ERK2-P	Asp-His-Thr-Gly-Phe-Leu-Thr-Glu-Tyr-Val-Ala-Thr-Arg-Trp-Cys (SEQ ID NO:10)	1.58	1.18	0.51	0.30	0.07	0.04
Myc Ser58/62-P	Glu-Leu-Leu-Pro-Thr-Pro-Pro-Leu-Ser-Pro-Ser-Arg-Arg-Ser-Cys (SEQ ID NO:11)	0.11	0.05	0.03	0.02	0.02	0.02
P38-2P	Leu-Ala-Arg-His-Thr-Asp-Asp-Glu-Met-Thr-Gly-Tyr-Val-Ala-Thr-Arg-Cys (SEQ ID NO:12)	0.54	0.30	0.08	0.08	0.04	0.04
JNK2P	Ser-Phe-Met-Met-Thr-Pro-Tyr-Val-Val-Thr-Arg-Tyr-Tyr-Arg-Cys (SEQ ID NO:13)	1.49	0.44	0.12	0.07	0.03	0.02